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ABSTRACT

Bacterial transformation is a commonly used technique in genetic engineering that involves transferring a gene of interest into a bacterial host so that the bacteria can be used to produce large quantities of the gene product. Although several kits are available for performing bacterial transformation in the classroom, students do not always clearly understand what they are doing by following the procedure. This document presents an exercise that uses paper DNA sequences to simulate the process of bacterial transformation and can be used in biochemistry, biotechnology, or any level biology class. In advanced biology classes it can be used to provide an introduction to the bacterial transformation laboratory while in general biology classes it can be used to help students understand this new technology. In the exercise students identify a gene of interest, choose a restriction enzyme to isolate the gene, construct a plasmid vector to carry the gene into bacterial cells, ligate the gene into the plasmid, then transcribe and translate the gene product. Key concepts include DNA structure and function, restriction enzymes, plasmid vectors, one gene-one polypeptide, transcription, and translation. (JRH)

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PAPER GENETIC ENGINEERING

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PAPER GENETIC ENGINEERING

RATIONALE: Genetic engineering encompasses techniques that have revolutionized the biotechnology industry and biological research. These techniques have made it possible to manipulate organisms at a genetic level, to move DNA from one organism to another, to amplify genes of interest and to study mechanisms of genetic control. Recent advances made possible by genetic engineering include the cloning and production of pharmaceutical products such as human insulin and human growth hormone and the development of recombinant DNA vaccines such as the Hepatitis B vaccine.

Bacterial transformation is a commonly used technique of genetic engineering that involves transferring a gene of interest (human insulin, for example) into a bacterial host so that the bacteria can be used to produce large quantities of the gene product. Although several kits are available for performing bacterial transformation in the classroom, students do not always clearly understand what they are doing by following this procedure. This exercise uses paper DNA sequences to simulate the process of bacterial transformation. It can be used in biochemistry, biotechnology or any level biology class. In advanced biology classes, it provides an introduction to the Advanced Placement bacterial transformation laboratory, and in general biology classes it works well on its own to help students understand this new technology. This exercise is quite flexible. It can be simplified for use in middle school classes, or made more complicated to challenge older students.

INTRODUCTION: In this exercise, students identify a gene of interest, choose a restriction enzyme to isolate the gene, construct a plasmid vector to carry the gene into bacterial cells, ligate the gene into the plasmid and then transcribe and translate the gene product. Questions to be answered at each step along the way reinforce key concepts and help students understand exactly what they are doing.

Key Concepts: DNA structure and function, restriction enzymes, plasmid vectors, one gene-one polypeptide, transcription, translation.

Materials: Each pair or group of students will need...

1 scissors
scotch tape
white paper DNA sequences
colored paper plasmid DNA sequences
transparency film restriction enzyme recognition sequences

DIRECTIONS:

The procedure for this exercise is fairly simple (in theory). You will:

1. *LOCATE* the gene coding for the protein you want to manufacture
2. *ISOLATE* the desired gene by using **restriction enzymes** to cut it out of the DNA
3. *OPEN UP* a plasmid (a small circular DNA molecule) which will carry the gene by cutting it with the same restriction enzyme
4. *INSERT* the gene into the plasmid (similar to splicing two pieces of tape together)
5. *TRANSFORM* host cells (bacteria) by inducing them to take in the plasmid carrying the gene
6. *SCREEN* the host cells to see which of them have taken in the plasmid and are producing the protein you want.

FOLLOW THE DIRECTIONS CAREFULLY AND BE SURE YOU KNOW WHAT YOU ARE DOING BEFORE YOU CUT OR TAPE ANYTHING.

PROCEDURE:

I. LOCATION OF TARGET GENE

1. The white pages contain strips of cellular DNA sequences. Carefully cut along the lines to separate these sequences into strips of double stranded DNA. DO NOT cut the numbers off the tops of the strips.

2. Assemble the strips into one long strand of DNA by taping the non-numbered end of each piece to the top of the next numbered piece (i.e., tape the non-numbered end of strip #1 to the top of strip #2, then connect the other end of strip 2 to strip 3 and so on...). Do not cover up any of the letters. You should end up with one long piece of DNA with only the number 1 showing at the top.

3. This strand of DNA contains the gene we are interested in. The strand of nucleotides on the LEFT will be referred to as the TEMPLATE strand, and this is the strand that will be used to produce mRNA during transcription. Transcription begins when an enzyme called RNA Polymerase binds to the following PROMOTER sequence: **A T A T T A.** In order for your plasmid to successfully produce the gene product, it must include a promoter sequence. This sequence can be part of the plasmid itself, or the gene to be inserted in the plasmid can include its own promoter. (Keep this in mind later when you are constructing your recombinant plasmid).

4. Scan the left-hand strand of your DNA for the PROMOTER sequence **A T A T T A.** Outline this sequence and label it "Promoter"

5. Although RNA polymerase binds to the promoter, the coding portion of the gene actually BEGINS at the START CODON: **TAC.** This codon can be found just

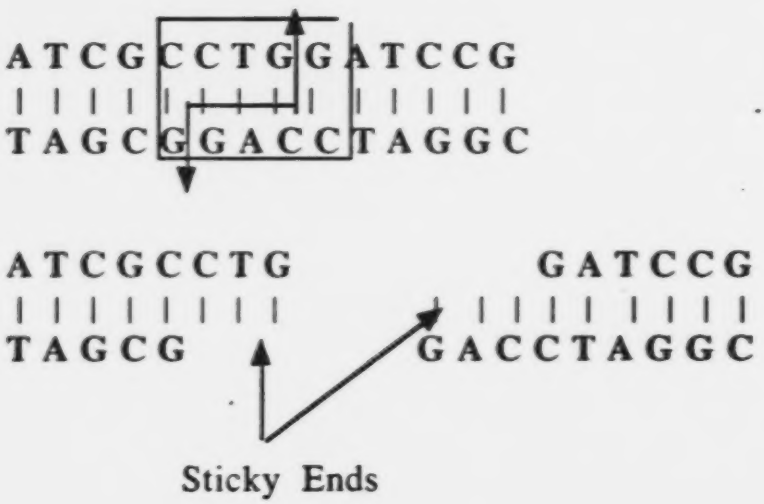
below the promoter on the cellular DNA. Find this codon and label it "start."

6. The TERMINATION sequence **C G G G C G** signals the end of the gene. Scan the left-hand strand of your DNA for the TERMINATION sequence **C G G G C G.** Outline this sequence and label it "Termination"

7. The length of DNA between these two sequences (including the sequences themselves) represents the gene we would like to isolate and engineer into the plasmid. **DO NOT CUT IT OUT YET.**

II. ISOLATION OF TARGET GENE

The target gene can be removed from the rest of the DNA by using **restriction enzymes**, enzymes that act like molecular scissors. These enzymes bind to and cut DNA at specific sequences, called recognition sequences. Each restriction enzyme has a different recognition sequence. The enzymes have funny names (like Ava II) that come from the organisms from which they were first isolated. The restriction enzymes you will use in this lab are found on pieces of transparency film. The dotted line running through the recognition sequence of each enzyme represents the line along which the DNA will be cut. Notice that this cut will produce "sticky ends", overhanging unpaired nucleotides which can be reattached if the complementary sticky ends are matched. See the example below:



Sticky Ends

Before deciding which enzyme(s) would be best to cut out the target gene, you must find the recognition sequences for all of the enzymes on your DNA. To do this, slide the transparency representing one enzyme along the entire length of the DNA strand until you find the sequence it matches. Outline each restriction site on the DNA and label it with the NAME of the enzyme that cuts there. Repeat this process for each of the six enzymes.

The enzyme used to isolate the gene must be able to chop out the entire gene, from above the start codon (TAC) to below the termination sequence. The enzyme to be used must NOT cut inside the gene (i.e., it must not cut between the start codon and the termination sequence). You have the following options in choosing which restriction enzyme to use:

OPTION 1: Use an enzyme that cuts above the promoter and below the termination sequence. If you choose this option, you do not need to ensure that the plasmid DNA contains a promoter sequence of its own. However, in "real life," plasmids will only accept inserts of about 4,000-5,000 base pairs, so you run the risk of creating an insert that is too large to be accepted by your plasmid if you include the cellular promoter.

OPTION 2: Use an enzyme that cuts *between* the promoter and the start codon (i.e., below the promoter and above the start codon) and below the termination sequence. If you choose this option, you must be sure that when you assemble your plasmid, the plasmid DNA contains a promoter sequence. The advantage of this option is that the cellular DNA fragment to be inserted in the plasmid is smaller and therefore it is more likely that successful insertion will occur.

REGARDLESS OF WHICH OPTION YOU CHOOSE, BE SURE THAT THE ENZYME YOU SELECT DOES NOT CUT BETWEEN THE START CODON AND THE TERMINATION SEQUENCE.

Look carefully at your labeled DNA strand and answer the following questions in the space provided:

1. Which restriction enzyme(s) would be BEST to use to isolate the target gene? Explain WHY your choice would be best.

2. Which enzyme(s) would NOT be useful? Explain why not.

III. PREPARATION OF THE PLASMID

Plasmids are small circular molecules of DNA that can serve as **vectors**, meaning that they can be used to carry the gene of interest from one organism to another. Before the gene you have isolated can be inserted into the plasmid, you must construct the plasmid and locate its origin of replication, restriction sites and antibiotic resistance genes.

1. Scan the plasmid sequences and identify the PROMOTER region: **A T A T T A.** Label this "promoter." Also, identify the origin of replication, the

kanamycin resistance gene and the ampicillin resistance gene. These genes will enable any cell that takes in this plasmid to grow in the presence of these antibiotics. Cells that do NOT take in the plasmid will NOT grow in the presence of ampicillin and kanamycin.

2. Cut out the strips of plasmid DNA from the colored sheets of paper. Assemble the strips end-to-end as you did with the cellular DNA, but this time you may tape them together in any order. You do not need to use ALL the pieces to assemble the plasmid, but your plasmid MUST contain an origin of replication and at least ONE of the antibiotic resistance genes. In addition, if your cellular DNA fragment does not have a promoter (because you chose option 2), the plasmid promoter must be included.

3. Attach the two ends of the strip to form a CIRCULAR plasmid.

4. Use the transparencies as you did before to locate and label the restriction enzyme recognition sites on the plasmid.

IV. INSERTION OF THE TARGET GENE INTO THE PLASMID

1. Decide which restriction enzyme(s) you want to use.The enzyme(s) you choose must cut the entire target gene out of the cellular (white) DNA. You must use this SAME enzyme(s) to open up the plasmid, and the plasmid's origin of replication and AT LEAST ONE antibiotic resistance gene must remain intact. You may use more than one enzyme, but keep in mind that whatever enzyme or combination of enzymes you use will cut ALL of its sites.

2. Carefully cut the cellular DNA, following the dotted line cutting pattern for the enzyme you have chosen. Cut ALL of the restriction sites for the enzyme you have chosen.

3. Cut the plasmid with the SAME enzyme(s) cutting pattern- this will open up the circular plasmid and create "sticky ends" that correspond to the ends of the cellular DNA.

4. Match up the sticky ends from the isolated gene with those of the plasmid and tape the gene into place in the plasmid. Be sure that you match complementary bases to each other (Match A to T and G to C)

5. Answer the following question in the space provided:

Why is it necessary to use the SAME restriction enzyme(s) to cut out the gene and to open the plasmid?

You have now constructed a plasmid that carries the target gene. The plasmid can be inserted into bacterial cells through a process called transformation, and the bacterial cells will transcribe and translate the plasmid DNA to produce the protein we want to manufacture.

V. EXPRESSION OF THE TARGET GENE

1. Locate the promoter region (A T A T T A) which is now part of the plasmid. Remember to look for this region on the LEFT hand strand of DNA. Just below this region, locate the START codon (T A C) on the cellular (white) DNA.

2. Starting with the start codon, write the sequence of the mRNA strand that would be transcribed from this DNA. Remember that an A on the DNA will be transcribed into a U on the mRNA, T on the DNA will be transcribed into a A on the mRNA, C on the DNA will be transcribed into a G on the mRNA, G on the DNA will be transcribed into a C on the mRNA. Stop transcribing when you reach the first base of the termination sequence (C G G G C G).

3. Write the sequence of the mRNA transcript below:

_____

_____

_____

_____

4. Separate the mRNA transcript into codons by placing a slash after every third letter, beginning with AUG/

5. Use a codon chart (which can be found in any biology textbook) to TRANSLATE the mRNA sequence into an amino acid sequence. Write the sequence of amino acids that would be produced from this mRNA on the line below:

_____

_____

_____

_____

_____

_____

_____

Answer the following questions in the space provided:

-What would happen if an extra G were inserted between the first codon (A U G) and the second codon? What effect would this have on the sequence of amino acids produced by translation?

-What would happen to the sequence of amino acids if the 5th mRNA codon were changed from AGA to AGG? What if this 5th codon were changed from AGA to GGA?

-What would happen if the 3rd mRNA codon was changed from UAU to UAG?

TEACHER NOTES

There are a variety of ways to modify this activity for use by students of different ability levels.

1. To simplify the search for an appropriate enzyme, reduce the number of enzymes students need to consider.

2. To make the problem more complex, add more enzymes. Have students consider the relative merits of using only one enzyme versus using a pair of enzymes to isolate the gene and open the plasmid. What are the advantages and disadvantages of each of these strategies?

3. Keep in mind that the way in which students assemble the plasmid sequences may cause some enzyme recognition sites to "appear" or "disappear." For this reason, each group's plasmid will differ slightly.

4. Cellular DNA is often protected from restriction enzyme digestion by methylation. Adding methyl groups to specific nucleotides at enzyme recognition sites prevents the enzymes from binding so they cannot cut the DNA. Modify the cellular DNA sequences so that some of the enzyme sites are methylated and therefore those enzymes are "unusable."

ADDITIONAL INFORMATION: The Finer Points of Genetic Engineering

This activity can be modified to make it more challenging for advanced students. Additional information about plasmid construction and genetic engineering strategies is provided below.

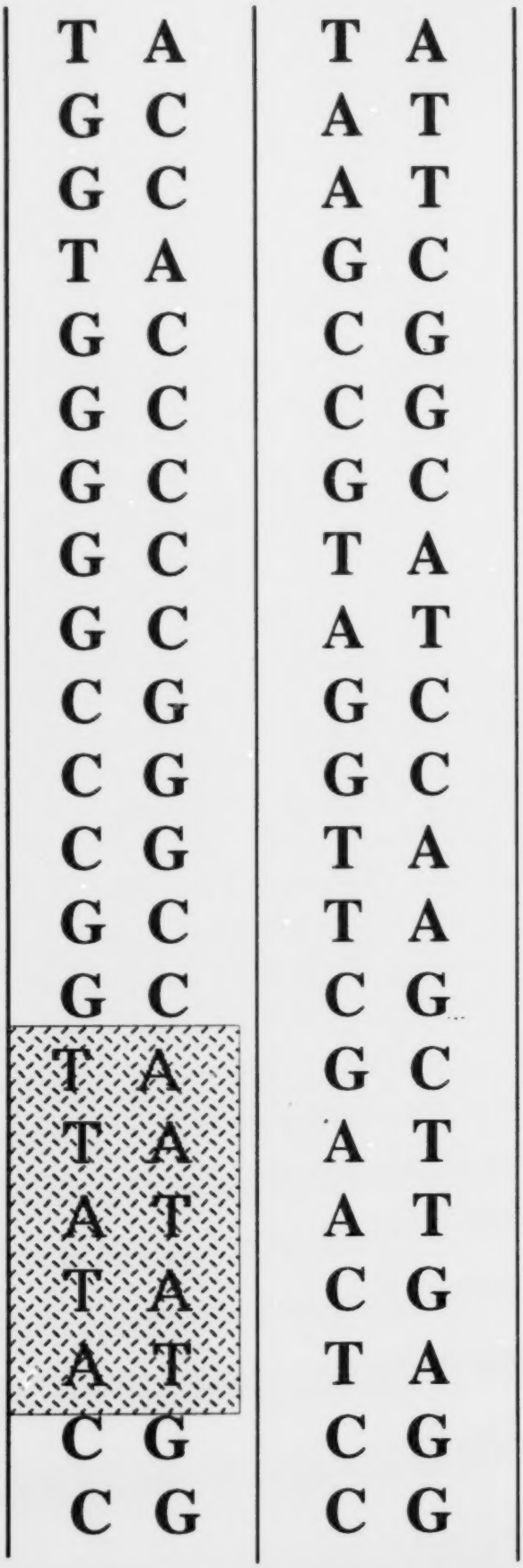
I. Single enzyme digests, efficiency of insertion and selection strategies

Using only one enzyme to cut out the target gene and open the plasmid may decrease the efficiency of insertion because the sticky ends of the cellular DNA are complementary to themselves, and the sticky ends of the plasmid are complementary to themselves. For this reason, three different outcomes are possible during ligation: 1) the desired outcome, sticky ends of target gene annealing to sticky ends of plasmid and ligating correctly to produce plasmid with inserted target gene 2) plasmid sticky ends re-annealing to themselves and ligating to produce closed plasmids without inserts and 3) sticky ends of the target gene annealing to themselves to produce small "loops" of target gene sequence that are not contained within a plasmid (and therefore will not be replicated). Which of these outcomes occurs depends on random interactions in the ligation mix, but formation products 2 and 3 certainly decreases the amount of desired product 1 that forms. In addition, one is now faced with the problem of distinguishing cells that take up plasmid WITH insert (product 1) from cells that take up plasmid WITHOUT insert (product 2).

This can be accomplished by employing both antibiotic resistance genes. A possible strategy might work as follows: choose an enzyme that cuts inside the kanamycin resistance gene but NOT in the ampicillin resistance gene. Plasmids that contain inserted target gene will have functional ampicillin resistance, but will have disrupted, non-functional kanamycin resistance. Plasmids that religate without insert will have both resistance genes intact and therefore will confer resistance to ampicillin and kanamycin on transformed cells. Following transformation, cells are grown first on ampicillin plates. This media allows both cells containing plasmid + target gene and cells containing plasmid only to grow. These colonies are then transferred onto 2 new plates: one containing ampicillin only and one containing ampicillin and kanamycin. This process is accomplished by using a sterile toothpick to move colonies one at a time onto the 2 plates in a grid pattern, so that cells from one colony are placed in the same spot on both amp and ampicillin+kanamycin plates. By comparing which colonies grow on these plates, one can distinguish colonies that carry plasmid + target gene (those that grow on ampicillin but NOT on kanamycin) from colonies that carry plasmid only (those that grow on BOTH ampicillin AND kanamycin).

(1)		(3)		(5)		(7)		(9)		(11)	
T	A	C	G	T	A	C	G	T	A	A	T
G	C	T	A	A	T	C	G	A	T	G	C
G	C	T	A	C	G	T	A	C	G	C	G
G	C	A	T	A	T	T	A	T	A	G	C
C	G	A	T	A	T	T	A	C	G	G	C
C	G	G	C	C	G	T	A	G	C	G	C
T	A	T	A	A	T	A	T	A	T	G	C
A	T	C	G	T	A	A	T	G	C	C	G
G	C	A	T	A	T	A	T	C	G	C	G
G	C	A	T	A	T	T	A	G	C	C	G
C	G	G	C	C	G	G	C	A	T	T	A
A	T	C	G	G	C	T	A	A	T	A	T
C	G	A	T	T	A	A	T	A	T	G	C
A	T	G	C	C	G	A	T	A	T	G	C
G	C	G	C	T	A	T	A	A	T	A	T

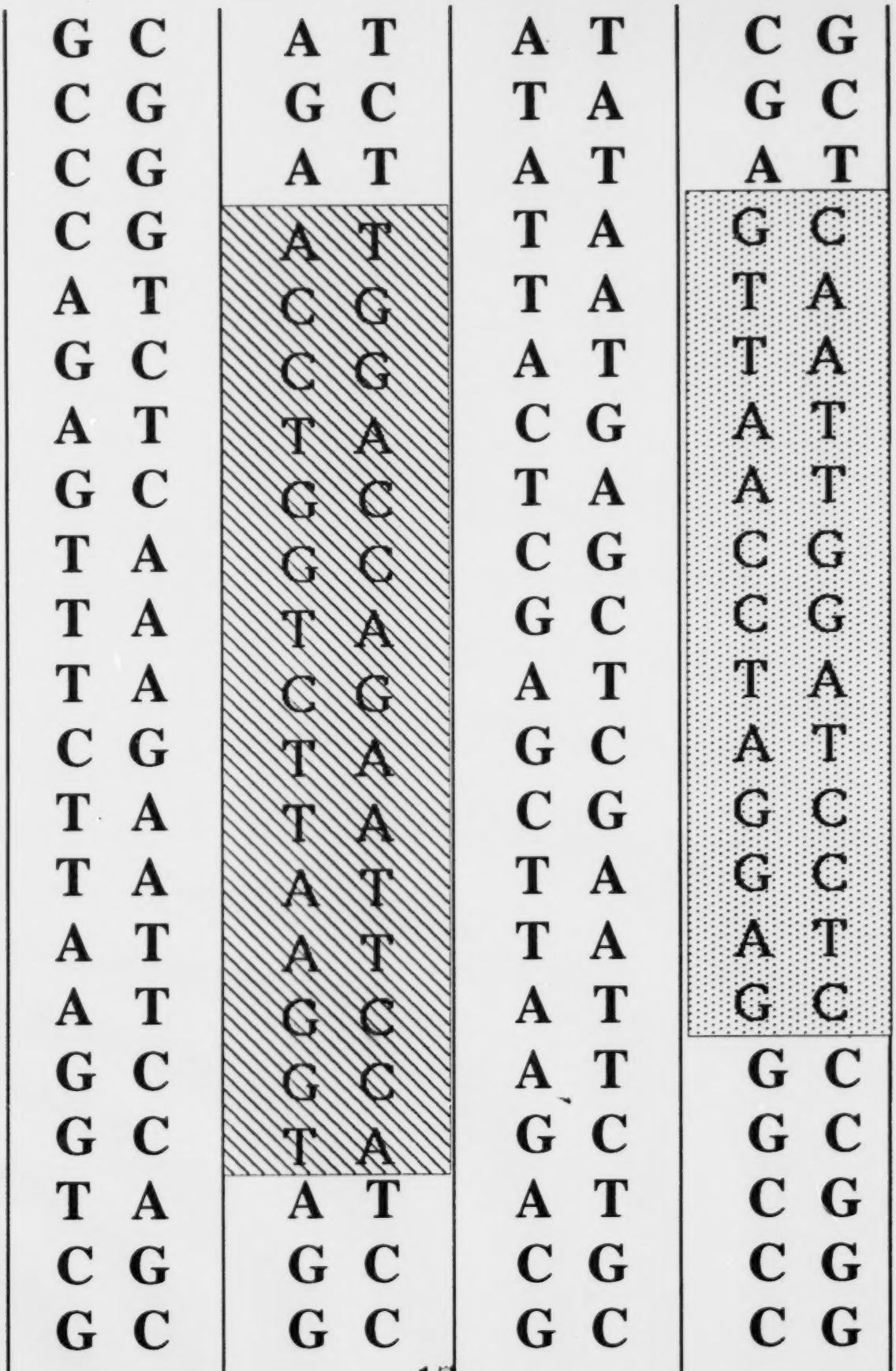
(2)	(4)	(6)	(8)	(10)
G C	T A	C G	A T	A T
G C	T A	T A	T A	C G
C G	A T	T A	T A	T A
C G	T A	C G	C G	T A
C G	A T	G C	C G	C G
G C	T A	T A	T A	G C
G C	T A	C G	C G	A T
A T	A T	A T	G C	A T
G C	C G	T A	G C	A T
A T	T A	T A	G C	A T
T A	C G	G C	C G	T A
T A	G C	G C	G C	C G
C G	A T	C G	G C	T A
A T	G C	C G	A T	T A
G C	A T	T A	T A	A T



ORIGIN OF REPLICATION

AMPICILLIN RESISTANCE

KANAMYCIN RESISTANCE



Enzymes

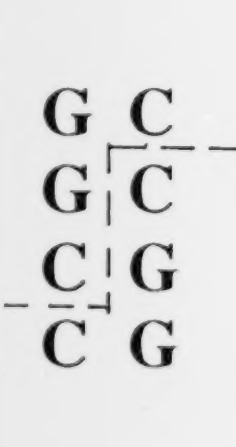
Ava II

Hin dIII

Sac I

Bam HI

Eco RI



Hpa II